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Autonomic modification of intestinal smooth muscle contractility

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Montgomery LE, Tansey EA, Johnson CD, Roe SM, Quinn JG. Autonomic modification of intestinal smooth muscle contractility. *Adv Physiol Educ* 40: 104–109, 2016; doi:10.1152/advan.00038.2015.—Intestinal smooth muscle contracts rhythmically in the absence of nerve and hormonal stimulation because of the activity of pacemaker cells between and within the muscle layers. This means that the autonomic nervous system modifies rather than initiates intestinal contractions. The practical described here gives students an opportunity to observe this spontaneous activity and its modification by agents associated with parasympathetic and sympathetic nerve activity. A section of the rabbit small intestine is suspended in an organ bath, and the use of a pressure transducer and data-acquisition software allows the measurement of tension generated by the smooth muscle of intestinal walls. The application of the parasympathetic neurotransmitter ACh at varying concentrations allows students to observe an increase in intestinal smooth muscle tone with increasing concentrations of this muscarinic receptor agonist. Construction of a concentration-effect curve allows students to calculate an EC_{50} value for ACh and consider some basic concepts surrounding receptor occupancy and activation. Application of the hormone epinephrine to the precontracted intestine allows students to observe the inhibitory effects associated with sympathetic nerve activation. Introduction of the drug atropine to the preparation before a maximal concentration of ACh is applied allows students to observe the inhibitory effect of a competitive antagonist on the physiological response to a receptor agonist. The final experiment involves the observation of the depolarizing effect of K^+ on smooth muscle. Students are also invited to consider why the drugs atropine, codeine, loperamide, and botulinum toxin have medicinal uses in the management of gastrointestinal problems.

intestine; acetylcholine; epinephrine; enteric nervous system

THIS PRACTICAL investigates the contraction of smooth muscle in the small intestine as well as the occurrence of spontaneous contractions and their modification by the autonomic nervous system (ANS). It also provides an introduction to several key concepts in receptor pharmacology. These are understanding the difference between receptor agonists and antagonists, constructing agonist concentration-effect curves, and distinguishing between competitive and noncompetitive antagonists. This is achieved by demonstrating the effect of Ach and epinephrine on tension generated by intestinal smooth muscle. The practical also demonstrates inhibition of the contractile effect of ACh by applying atropine, a competitive antagonist at the muscarinic type of cholinoceptor.

Background

Smooth muscle in the small intestine contracts rhythmically in the absence of neuronal or hormonal stimulation; such contractions are referred to as phasic (14). These phasic contractions are initiated by the activity of a particular cell type, the interstitial cell of Cajal (ICC) (9), which is distinct from smooth muscle cells. These cells form a network between and within the circular and longitudinal muscle layers of the intestinal wall (1). ICCs that lie in the myenteric region (i.e., between muscle layers) initiate depolarization and pacemaker potentials, referred to as slow waves (6). These waves generate the basic electrical rhythm for phasic contractions and are always there whether contraction occurs or not (13). They occur at different frequencies at various points along the gastrointestinal (GI) tract (13), and these frequencies can range from a few to >30 cycles/min depending on species (7). Slow waves set the maximum frequency at which contraction can occur at a particular site, with individual slow waves not necessarily resulting in muscle contraction. For contraction to occur, a spike potential must be generated by smooth muscle cells, seen as transient membrane depolarizations superimposed on the plateau phase of the slow wave (4). Spikes are believed to be generated, at least in part, by inward Ca^{2+} currents (8). The basic contractile activity that occurs due to the occurrence of slow waves and spikes is modulated by the enteric nervous system (ENS) (4). This is the collection of neurones within the intestinal wall that directly modulates intestinal muscle contractions (13). The ANS, including sympathetic and parasympathetic nerves, modulates contractile activity indirectly through the ENS (5).

ACh is the major excitatory neurotransmitter of the ENS, and its excitatory effect on intestinal smooth muscle is mediated through the muscarinic type of cholinoceptor (4). ACh also mediates the excitatory effects of parasympathetic nerves that act on intestinal smooth muscle indirectly through an effect on the ENS (4). ACh has been shown to increase the amplitude of spontaneous contractions in the rabbit small intestine, and the frequency of these contractions in the circular but not longitudinal muscle layers (3). The muscarinic subtype of receptor that directly mediates smooth muscle contraction in the GI tract is the M_3 subtype (12). This subtype is coupled to G_q and the activity of PKC (2).

Sympathetic nerves of the ANS also modulate GI tract motility indirectly through the ENS, having an inhibitory effect on motility (5). This inhibition occurs through two different mechanisms. Release of norepinephrine acts presynaptically to decrease activity in the cholinergic nerves of the ENS; this is through activation of the α_2 adrenoceptor subtype (15). This subtype of adrenoceptor is coupled to G_i and inhibition of adenylyl cyclase (10). Norepinephrine also acts directly on intestinal smooth muscle cells to cause relaxation through activation of β_3-adrenoceptors, which are coupled to G_s and PKA (11).

Learning Objectives

After completing this activity, the student should be able to:

- Describe the difference between a receptor agonist and antagonist.

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- Show experimentally, through the use of tension recordings, the effect of ACh on tension generated by the rabbit small intestine.
- Use these recordings to construct a concentration-effect curve for the receptor agonist ACh.
- Show experimentally, through the use of tension recordings, how application of the competitive antagonist atropine affects the intestinal response to ACh.
- Describe the difference between a competitive and noncompetitive antagonist and be able to draw hypothetical concentration-effect curves to illustrate this.
- Show experimentally, through the use of tension recordings, the effect of the receptor agonist epinephrine on tension generated by the rabbit small intestine.
- Show experimentally, through the use of tension recordings, the effect of application of a high-K^+ solution on tension generated by the rabbit small intestine.

Activity Level

We currently use this practical to teach level 1 and 2 undergraduate students from Biomedical Science, Dentistry, Human Biology, Medicine, and Pharmacy degree pathways. Some of the students on the Medicine and Dentistry degree pathways have entered the course as graduates.

Prerequisite Student Knowledge

Before completing this activity, students should have a basic knowledge of the ENS and ANS. They should be able to identify the main neurotransmitters released from the postganglionic neurones of sympathetic and parasympathetic nerves and should be able identify epinephrine as a hormone released from the adrenal glands and as an adrenoceptor agonist. They should also be able to identify the types of cholinoceptors and adrenoceptors expressed by mammalian intestinal smooth muscle and associated nerves. Finally, they should have an appreciation of how depolarization of the muscle cell membrane occurs and how this can evoke a contractile response.

Time Required

It takes ~10–15 min for students to prepare the segment of intestinal smooth muscle for tension recording and familiarize themselves with the drugs and solutions they have been supplied with. The experiments take ~45–60 min to complete, and construction of the concentration-effect curve takes another 30–45 min.

METHODS

Equipment and Supplies

The following equipment and supplies are needed:

- A segment of the rabbit small intestine of ~3 cm long and freshly dissected. The guinea pig small intestine has also been used successfully in our laboratory, but using rabbit small intestine confers specific advantages. Fewer animals are required to provide enough tissue for large classes, and the larger diameter of the small intestine from the rabbit makes it easier to handle and set up for recording.
- A tension transducer (range of 0–50 g) to connect to a computer with basic data-acquisition software. This should be capable of displaying a simple graph of tension against time and marking drug additions. There are commercially available computer-based analog-to-digital converter and display systems (e.g., LabTutor, AD Instruments, and Dunedin). Currently, we use equipment and software that have been manufactured on site and adapted for display on personal computers.
- An organ bath (with a 50-ml level indicator) to support the tension transducer and keep the tissue in a warm oxygenated environment. The temperature of the organ bath is set to 38°C to offset cooling from the top of the bath; this keeps the temperature of the saline inside at ~37°C. The bath is oxygenated by bubbling the saline with 100% O_2.
- A combination of thread and spring clips to connect the segment of the gut to the tension transducer.
- The following drugs at the given concentrations:

  1. ACh (chloride): 0.05 mmol/l (9 mg/l)
  2. ACh (chloride): 0.5 mmol/l (91 mg/l)
  3. Epinephrine (bitartrate): 0.5 mmol/l (166 mg/l)
  4. K^+ (KCl): 1 mol/l (74.5 g/l)
  5. Atropine (sulphate): 0.05 mmol/l (35 mg/l)

- Syringes (1 ml with 21-gauge needles). The needles have had their tips removed (using pliers) and blunted for safety (using a file).
- Physiological saline solution (containing 146 mmol/l NaCl, 3 mmol/l KCl, 12 mmol/l$NaHCO_3$, 1 mmol/l $MgCl_2$, 0.5 mmol/l NaH_2PO_4, 1.5 mmol/l $CaCl_2$), and 5 mmol/l D-glucose.

Ethical Approval

In the United Kingdom, no specific ethical approval is required as long as the rabbits are euthanized in accordance with the United Kingdom Home Office Inspectorate (The Use of Animals in Scientific Procedures Act, 1986). The method used in this institution is anesthetization using CO_2 followed by cervical dislocation.

Instructions

Setting up the preparation. The experimental setup is shown in Fig. 1. Students are advised to wear a white laboratory coat, glasses, and gloves throughout the practical, to avoid potential contamination with bacteria from intestinal contents or blood-borne pathogens. The length of the gut should be transferred from the animal to the organ bath as quickly as possible to avoid deterioration of the tissue. Care needs to be taken when connecting the tissue to the tension transducer. We recommend that the

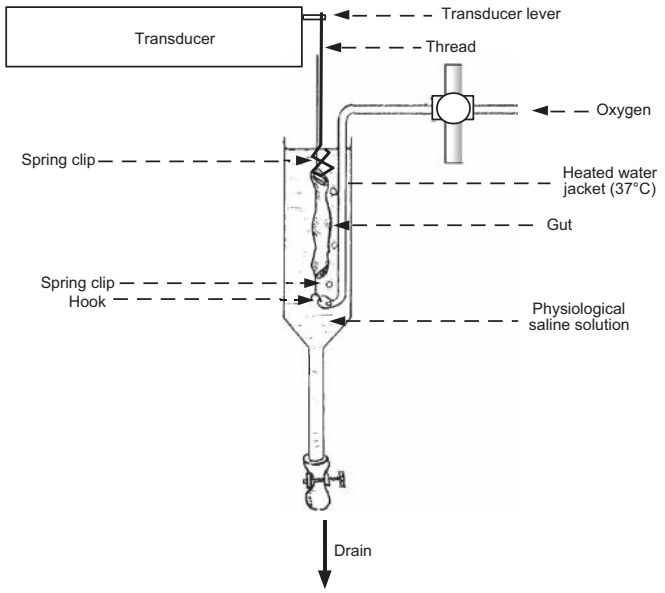

Fig. 1. Arrangement of the organ bath, tissue, and pressure transducer.

Table 1. *Drug additions required to create a range of ACh concentrations in the organ bath*

Concentration Required in the Bath, μM	Add Volume From 0.05 mmol/l ACh Stock Solution	Add Volume From 0.5 mmol/l ACh Stock Solution	Total Contraction, g
0.1	0.1 ml		
0.2	+0.1 ml		
0.5	+0.3 ml		
1	+0.5 ml		
2	Continue →	+0.1 ml	
5		+0.3 ml	
10		+0.5 ml	
20		+1 ml	
50		+3 ml	
100		+5 ml	

students connect it up with some slack in the thread and tighten it up until ~5 g of tension are placed on the gut. The tension graph should then be zeroed for the start of the experiments.

Experiment 1: effect of ACh on tension generated by the rabbit small intestine. To begin the experiment, record ~1 min of baseline contractions. Using a syringe, draw 1.0 ml of the more dilute ACh solution (0.05 mmol/l), making sure it contains no bubbles. Insert the tip of the needle into the fluid in the bath without touching the gut. Eject the first 0.1 ml of drug and pause for ~20 s to observe any effect. The bubbling O_2 should mix the solution. Wait until the effect has stabilized before adding another dose. Table 1 shows how much of each stock solution should be added to create a range of concentrations in the bath. Mark the recording to show the timing of each drug addition. Print these results and clear the record in preparation for *experiment 2*.

Before the next experiment, empty the bath and immediately refill it with fresh warm physiological saline solution. After 2 min, wash out the bath a second time, and wait for a new baseline to be established.

Experiment 2: effect of epinephrine on intestinal smooth muscle contraction. Identify the lowest concentration of ACh that produced the maximum contractile effect in *experiment 1*. Determine how much of each stock solution is required to create this concentration in the organ bath, taking into account that the calculations used to create Table 1 were based on ACh accumulating in the bath. Record 1 min of baseline and add the single dose of ACh that had a maximal effect. Mark and label the addition. Wait for the change in tension to equilibrate, and with the ACh still present, add a single dose of 0.5 ml of the epinephrine stock solution (to create a final concentration in the bath of 5 μmol/l). When relaxation has occurred and a new baseline established, print the results and clear the record in preparation for *experiment 3*. Wash the drugs out of the bath with two changes of physiological saline solution. Do not begin the next experiment until the tissue begins to contract rhythmically.

Experiment 3: competitive inhibition of the contractile effect of ACh by the muscarinic antagonist atropine. Record ~1 min of baseline contractions and add 0.1 ml of the atropine stock solution (to create a final concentration of 0.1 μmol/l in the bath), marking the event. Wait for ~1 min and add the single dose of ACh that had a maximal effect (determined from *experiment 1*). Mark and label this addition and observe any changes. Complete recordings by adding 2.5 ml of the high-K^+ solution (to give a final concentration of 50 mmol/l K^+ in the bath). Observe the effects of this for a further 1 min.

Construction of the Concentration-Effect Curve

The recording made during *experiment 1* is used to generate a graph of tension (*y*-axis) against the concentration of ACh (*x*-axis). The *x*-axis should have a log scale. When determining the overall tension achieved after each drug addition, a line should be drawn across the middle of the individual contractile responses after each ACh addition (see Fig. 2). Ideally, this would give a sigmoidal shaped curve.

Troubleshooting

There are four common points of difficulty.

1. Without emphasis on how the tissue should be handled, it is not uncommon to see students leaving the gut sitting in physiological saline solution, which is rapidly cooling down and not being perfused with O_2.

2. Students often have difficulty applying the correct amount of tension on the segment of the rabbit gut. The thread should be taut enough to register contractions with the tension transducer but not so tight as to damage the contractile machinery of the tissue. We have found that ~5 g of tension on the preparation works well.

3. Incorrect addition of the single dose of ACh that had a maximal effect. It is useful to emphasize and explain verbally that the calculations for ACh addition shown in Table 1 take into account accumulation of the drug in the organ bath. This means that they cannot just read across a table entry; they need to add up all the doses that came before it to achieve the correct concentration.

4. The concentration-effect curve is not sigmoidal. We supply our students with semi-log graph paper, but if they use a linear scale for plotting ACh concentrations, the curve is unlikely to be sigmoidal. In addition, it is possible that their particular section of the gut would require a different set of ACh concentrations to be applied to see this shape. To achieve the plateau at either end of the graph, no significant

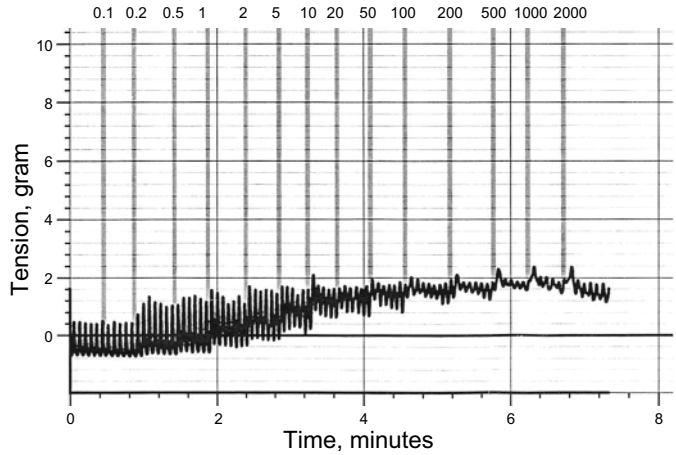

Fig. 2. Tension (in g) generated by the rabbit small intestine in response to increasing concentrations of ACh (in μmol/l). The concentrations of ACh used (in μmol/l) are shown above the record, and the vertical lines indicate the timing of these additions. The zero line on the y-axis shows the zero reference tension. The change in muscle tone should be measured from this zero line to an average tension generated by the muscle (which can be estimated "by eye") rather than the peak of individual contractions.

change in muscle tone with the beginning two and end two ACh additions should be recorded in *experiment 1*.

Safety Considerations

There are no special safety concerns for this practical; following general laboratory safety rules and wearing white coats, gloves, and safety glasses are sufficient.

RESULTS AND DISCUSSION

Experiment 1: Effect of ACh on Tension Generated by the Rabbit Small Intestine

When increasing concentrations of ACh are applied, the overall tension generated by the intestinal smooth muscle should increase. Ideally, at the smallest concentrations, it would take a couple of additions at the start to see any significant increase in tone, and, at the higher concentrations, at least two additions should evoke maximum tension, after which no further increase in tone will be observed. Individual contraction heights decrease at higher concentrations, as overall muscle tone increases and the muscle relaxes less. The frequency of individual contractions would not be expected to change (see Fig. 2).

If the tension values from *experiment 1* are plotted against ACh concentrations on a log scale, the expected sigmoidal shape is seen (see Fig. 3). We require our students to draw a plot by hand on graph paper we supply, joining the points with straight lines. However, students could also be asked to generate a plot fitted with a four-parameter logistic function, using statistical software such as GraphPad Prism. This is how the curve shown in Fig. 3 was generated. We also require our students to calculate the estimated concentration of ACh that generates 50% of the muscle's maximum response (EC_{50}). However, depending on their statistical knowledge, students could also be asked to calculate the goodness of fit (r^2) of the plot and collect class data to be able to determine mean values and SEs.

Experiment 2: Effect of Epinephrine on Tension Generated by the Rabbit Small Intestine

The muscle should be precontracted by applying the concentration of ACh that has a maximal effect, as determined from *experiment 1*. The application of epinephrine to the contracted intestine should show muscle relaxation, i.e., a decrease in overall muscle tone, as shown in Fig. 4.

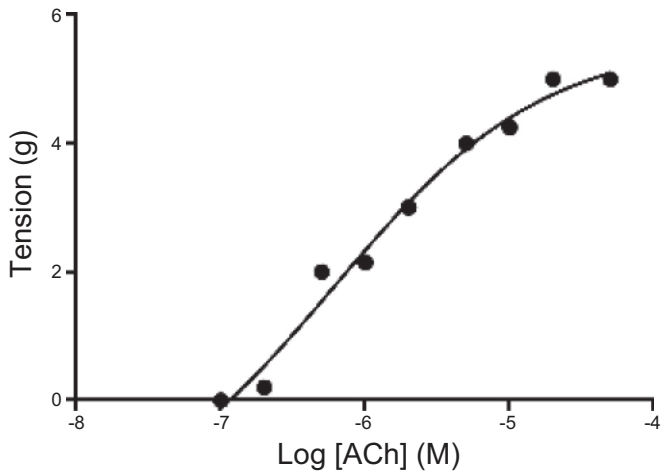

Fig. 3. Concentration-effect curve illustrating the excitatory effect of ACh on intestinal muscle tone.

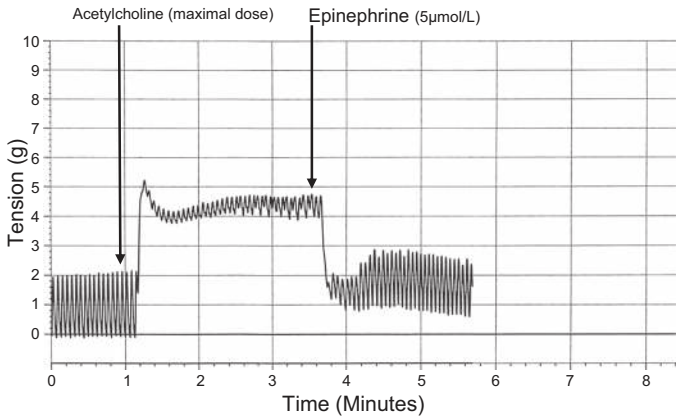

Fig. 4. Tension (in g) generated by the rabbit small intestine in response to the application of ACh and the reversal of this effect by the application of epinephrine. The concentrations of each agent used are shown above the record, and arrows indicate the timing of these additions.

Experiment 3: Competitive Inhibition of the Contractile Effect of ACh by the Muscarinic Antagonist Atropine

The addition of atropine (0.1 μmol/l) alone has no apparent effect on spontaneous contractions of the tissue. When the concentration of ACh that elicited a maximal response from the tissue is applied in the presence of atropine, the response should be reduced or completely absent (as shown in Fig. 5). After exposure to high-K^+ solution, the resulting membrane depolarization results in a large transient contractile response from the intestine.

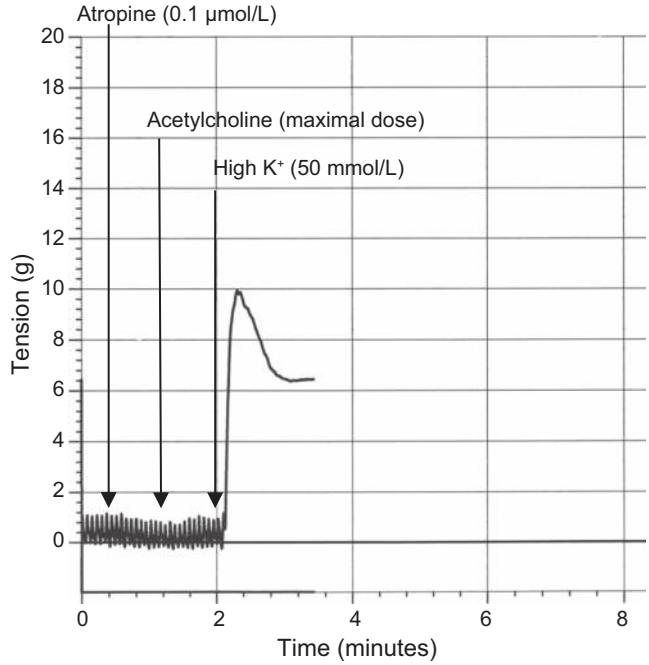

Fig. 5. Effect of atropine on ACh-mediated contractions in the rabbit small intestine and the application of high-K^+ solution. The concentrations of each agent used are shown above the record, and arrows indicate the timing of these additions.

Evaluation of Student Work

In addition to the construction of a concentration-effect curve on logarithmic paper, students should also be able to answer some basic questions to determine their conceptual understanding.

Experiment 1. How does ACh contribute to intestinal motility, digestion, and absorption?

ACh release mediates the excitatory effects of nerves of the ENS and parasympathetic nerves, increasing muscle tone. This increases the occurrence of segmentation in the intestine, mixing intestinal contents to help with mechanical digestion and enhancing contact of the chyme with intestinal walls to increase absorption.

Medicines that directly stimulate the myenteric plexus (e.g., senna) can increase GI tract motility. Using this information, can you suggest a condition that these drugs are useful in treating?

Stimulation of GI tract motility can improve bowel function in the condition of constipation.

The sigmoidal shape of the concentration-effect curve can be loosely linked to ACh receptor occupancy. Why is this link only a loose one?

Generally speaking, as the concentration of ACh increases, so does the occupation and activation of muscarinic receptors. However, we are measuring a physiological response (muscle contraction), which is not necessarily proportional to receptor occupancy or activation.

Calculate the EC_{50} value (i.e., the concentration that produces a 50% maximum response). What is the significance of this value?

The EC_{50} value is worked out by finding the point on the *y*-axis that correlates to 50% of the muscle's maximum response, drawing a line to the graph, and correlating it with the appropriate concentration on the *x*-axis. This is useful if you want to compare drugs that act on the same variable. For example, if the EC_{50} value of *drug A* is higher than that of *drug B*, then we would say that *drug A* is the less potent drug; in other words, you need more of it to produce the same effect. Potency depends on both the affinity of a drug for the receptor as well as its efficacy. It is important to note that the usefulness of the concentration-effect curve is limited to making comparisons between drugs; they cannot be used to directly determine the affinity or efficacy of a drug.

Experiment 2. How might epinephrine affect intestinal motility and therefore digestion and absorption, and where would it originate in the intact animal?

Epinephrine (released from the adrenal medulla) would activate the same adrenoceptors as norepinephrine does on smooth muscle cells and postganglionic parasympathetic fibers. Therefore, the effect of application of epinephrine would be the same as sympathetic nerve activation, i.e., a reduction in smooth muscle tone and mechanical digestion and absorption.

Experiment 3. How does the use of atropine enable you to attribute the action of ACh to a particular receptor subtype (i.e., muscarinic or nicotinic)?

Atropine specifically antagonizes the muscarinic type of cholinoceptor; it does not affect the nicotinic receptor. Therefore, if the response to ACh is completely absent in the presence of atropine, the response can be directly linked to the muscarinic rather than the nicotinic type of cholinoceptor.

How does the action of a competitive antagonist like atropine differ from a noncompetitive antagonist at the muscarinic receptor?

A competitive antagonist binds to the receptor at the same site as the agonist. The noncompetitive antagonist binds to a different site on the receptor to the agonist but still blocks transformation of the receptor to its activated state.

Why does the application of high-K^+ solution cause a transient contractile response from the tissue when it is unresponsive to ACh?

The response to ACh requires a receptor to mediate its contractile effect; K^+ directly depolarizes the cell membrane by adjusting the concentration of K^+ on either side of the membrane. A depolarization can be verified using the Nernst equation.

Inquiry Applications

Given how atropine affects ACh-mediated contractions of intestinal smooth muscle, can you think of how this action might have a medical use? (Antispasmodic drugs.)

Codeine and loperamide are both opiate drugs used for the symptomatic relief of diarrhea; they act to reduce secretion of intestinal fluid. What other physiological effect would make these drugs useful for this purpose? (Reduction in propulsive activity of the intestines.)

When morphine is used to achieve pain relief in a clinical setting, how might a patient's GI tract function be affected? As a result, which side effect would a patient's care providers be concerned about occurring? (Morphine decreases the propulsive activity of the intestines, and constipation can occur.)

Botulinum toxin produced by *Clostridium botulinum* inhibits ACh release from presynaptic nerve endings. Can you think of how this might be useful in the treatment of conditions affecting the GI tract? (As a treatment for conditions that result from intestinal overactivity, e.g., esophageal sphincter of achalasia and anal fissure.)

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: L.E.A.M. conception and design of research; L.E.A.M. performed experiments; L.E.A.M. prepared figures; L.E.A.M. drafted manuscript; L.E.A.M., E.A.T., C.D.J., S.M.R., and J.G.Q. approved final version of manuscript; E.A.T., C.D.J., S.M.R., and J.G.Q. interpreted results of experiments; E.A.T., C.D.J., S.M.R., and J.G.Q. edited and revised manuscript.

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